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Biochemical and functional characteristics of the human leukocyte membrane antigen family LFA-1, Mo-1 and p150,95*

The human leukocyte function-associated (LFA-1) antigen, the monocyte differentiation antigen Mo-1 which is characterized as the C3bi receptor and the glycoprotein p150,95 are characterized biochemically. Immunoprecipitations carried out with 6 different monoclonal antibodies (mAb) against LFA-1 indicated that four mAb (SPV-L1, SPV-L5, SPV-L7 and SPV-L11) were directed against the α chain, whereas mAb CLB54 and MHM-23 were found to react with the common β chain of LFA-1, Mo-1 and p150,95. LFA-1 and Mo-1 expressed on KG-1 cells or lymphocytes, monocytes and granulocytes from one donor were homogeneous. Interestingly the α chain of p150,95 showed heterogeneity. The molecular weight of the α chain expressed on monocytes was consistently higher than that of the α chain on granulocytes. The β subunits of LFA-1 and Mo-1 (as detected by mAb Bear-1) are not only similar in molecular weight and isoelectric focusing patterns, but it is demonstrated here that they are also identically glycosylated and have similar protein backbones as judged by tryptic peptide mapping. In spite of their structural similarities, LFA-1 and Mo-1 differ completely in some of their biological functions. Anti-LFA-1 mAb strongly inhibited monocyte-dependent T cell proliferation induced by tetanus toxoid or *Helix pomatia* hemocyanin and pokeweed mitogen-driven specific antibody production *in vitro*, whereas the anti-Mo-1 antibody Bear-1 was ineffective. These results suggest that the differences in these biological functions of LFA-1 and Mo-1 may be related to their different α subunits, which may recognize specific counter structures.

1 Introduction

The human leukocyte function associated (LFA-1) antigen, the monocyte differentiation antigen Mo-1 which is characterized as the C3bi receptor and the glycoprotein termed p150,95 belong to a family of related human leukocyte membrane antigens. LFA-1 is expressed on lymphocytes, monocytes and granulocytes [1], whereas Mo-1 and p150,95 are present on monocytes, granulocytes and "null" cells [2]. Recently, it has been shown that these molecules contribute to the adhesion reaction of these cells. Blocking experiments with anti-LFA-1 monoclonal antibodies (mAb) demonstrated that LFA-1 inhibits the adhesion step between effector and target cells in cytotoxic T lymphocyte, natural killer and lectin-dependent cellular cytotoxicity [1, 3, 4]. LFA-1 also participates in monocyte-dependent lectin-induced T cell proliferation suggesting that it also acts as an adhesion molecule between monocytes and T cells.

Mo-1, which is the receptor for the inactivated form of C3b, mediates the adhesion of soluble complexes or particles selectively opsonized with C3bi [5, 6]. The third member of this family, p150,95, is less well investigated but studies in patients with an inherited LFA-1, Mo-1 and p150,95 deficiency syndrome indicated that this molecule, probably similarly to LFA-1 and Mo-1, is associated with the adhesion of granulocytes [7]. In addition to their functional similarities these antigens are also structurally related. Each antigen is composed of an α and β subunit which are noncovalently associated. The β chains of all three molecules have a molecular mass (MM) of 95 kDa and have been shown to be similar [8]. The α chains of LFA-1, Mo-1 and p150,95 have different MM of 170, 165 and 150 kDa, respectively.

In the present study, 6 different mAb directed against the α or β chain of LFA-1 were used for the biochemical characterization of LFA-1, Mo-1 and p150,95 expressed on the human myeloblastic cell line KG-1. In addition, the heterogeneity of these molecules expressed on lymphocytes, monocytes and granulocytes derived from one donor was investigated. Our findings indicate that only the α chain of p150,95 showed heterogeneity. Finally we extended the findings of Sanchez-Madrid et al. [8] by demonstrating that the β subunits of LFA-1 and Mo-1 in addition to their similarities in MM and isoelectric focusing (IEF) patterns were identically glycosylated and had similar protein backbones as judged by tryptic peptide mapping. In spite of these structural similarities and the fact that both molecules act as adhesion molecules on granulocytes, it is reported here that both molecules differ completely in other biological functions. Anti-LFA-1 antibodies strongly inhibited monocyte-dependent antigen-specific T cell proliferation and pokeweed mitogen (PWM)-driven specific antibody production *in vitro*, whereas the anti-Mo-1 antibody, Bear-1, produced in our laboratory was ineffective.

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Abbreviations: HPH: *Helix pomatia* hemocyanin IEF: Isoelectric focusing LFA-1: Leukocyte function-associated antigen-1 MM: Molecular mass mAb: Monoclonal antibody(ies) NMS: Normal mouse serum SDS: Sodium dodecyl sulfate PAGE: Polyacrylamide gel electrophoresis pfc: Preformed complex PWM: Pokeweed mitogen TPA: 12-O-tetradecanoyl phorbol-13-acetate TT: Tetanus toxoid Endo-F: Endo- β -N-acetyl-glucosaminidase F ELISA: Enzyme-linked immunosorbent assay RAM: Rabbit anti-mouse

2 Materials and methods

2.1 Cells

Human leukocytes were isolated from a buffy coat from blood of healthy volunteer donors by centrifugal elutriation [9]. Monocytes, or monocyte subsets and granulocytes obtained in this way, were >95% pure, whereas lymphocytes were obtained at a purity of 99.9% [9]. The myeloblastic cell line KG-1 [10] was maintained in RPMI 1640 containing 10% fetal calf serum, sodium pyruvate (50 mg/ml), L-glutamine (2×10^{-3} M) and penicillin (100 IU)/streptomycin (100 µg/ml). This cell line was kindly provided by Dr. Robert F. Todd III (Dana-Farber Cancer Institute, Boston, MA). KG-1 cells were stimulated with 10 ng/ml 12-O-tetradecanoyl phorbol-13-acetate (TPA, batch 028, Midland Corporation, NY) for three days and restimulated every third day with 5 ng TPA. In this way adherent macrophage-like cells were obtained [11].

2.2 mAb

The mAb SPV-L1, SPV-L3, SPV-L5, SPV-L7 and SPV-L11 were obtained by immunization of BALB/c mice with cells of the T4⁺T8⁻ cytotoxic T lymphocyte clone HG-38 [12]. The antibodies were selected for inhibition of T cell-mediated killing by screening of the hybridoma supernatants 10–14 days after the fusion. CLB54 (LFA-1/1) was selected in a similar way, and is directed against the β chain of LFA-1, Mo-1 and p150,95 [13]. Anti-Mo-1, reactive with human monocytes, null cells, granulocytes, bone marrow cells and malignant myeloid cells [2, 14], was a gift from Dr. Robert F. Todd III (Dana Farber Cancer Institute, Boston, MA). Bear-1, Rupi-1 and Rupi-5 were obtained by immunization of BALB/c mice with human monocytes isolated by centrifugal elutriation. Bear-1 precipitated a 165-95-kDa complex and was found to be similar to Mo-1 (not shown). Rupi-1 reacts with a 35-kDa myeloid differentiation antigen expressed on human monocytes, granulocytes and myeloid leukemia cells. mAb Q5/13 is directed against HLA-DR and was kindly provided by Dr. Soldano Ferrone (New York Medical College, Valhalla, NY). MHM23 which is directed against the β chain of LFA-1 was a gift of Dr. A. McMichael (Nuffield Dept., John Radcliffe Hospital, Oxford, GB).

2.3 Radiolabeling and immunoprecipitation

Cell surface radioiodination with Na¹²⁵I (ICN Radiochemicals, Irvine, CA) was catalyzed by 1,3,4,6-tetrachloro-3 α , 6 α -diphenylglycoluril (Iodogen, Pierce Chemical Co., Rockford, IL) [15]. For immunoprecipitation, radiolabeled cells were lysed with 1% Nonidet-P40 in 0.01 M triethanolamine-HCl, pH 7.8, 0.15 M NaCl, 1 mM phenylmethylsulfonyl fluoride, 0.02 mg/ml ovomucoid trypsin inhibitor (Sigma Chemical Co., St. Louis, MO; TEA/NaCl buffer). Nuclear debris was removed from the lysates by centrifugation at 13 000 \times g for 15 min at 4°C. Material insoluble in the lysis buffer was removed by centrifugation at 100 000 \times g for 30 min in an air-driven centrifuge (Beckman Instruments, Palo Alto, CA). Lysates were precleared further by successive incubations with formalin-fixed *Staphylococcus aureus* bacteria (strain Cowan I) and preformed complex of mouse Ig and rabbit anti-mouse Ig, as described previously [16]. Precleared lysates were incubated for 3–4 h with a preformed complex made with the

specific mAb and rabbit anti-mouse Ig, and immunoprecipitates were removed from the lysates by centrifugation at 13 000 \times g. Precipitates were resuspended in 0.2 ml TEA/NaCl buffer with 0.5% sodium deoxycholate and spun through a discontinuous gradient consisting of 0.4 ml of 10% sucrose, 0.5% Nonidet-P40 in TEA/NaCl buffer, and 0.8 ml of 20% sucrose in the same buffer without detergents, at 13 000 \times g for 15 min. Subsequently, immunoprecipitates were washed once in 0.01 M TEA-HCl, pH 7.8, 0.2% Nonidet-P40.

2.4 Enzyme treatment

Endo- β -N-acetylglucosaminidase F (Endo-F) was kindly supplied by Drs. John Elder and Stephen Alexander (Scripps Clinic, La Jolla, CA). Endo-F treatment was performed directly on the immune complex. The protein was resuspended in 0.1 M sodium phosphate buffer, pH 6.1, containing 50 mM EDTA, 1% 2-mercaptoethanol, 0.1% sodium dodecyl sulfate (SDS) and boiled for 2 min. Then, Nonidet-P40 was added to 1% and samples were incubated with Endo-F for 2 h at 37°C, as described by Elder and Alexander [17].

2.5 Electrophoresis and autoradiography

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out on discontinuous vertical slab gels according to a modification of the Laemmli procedure [18]. Gradient gels were made either 5–15% or 10–15% in acrylamide. Two-dimensional gel electrophoresis was done in accordance with the method of O'Farrell [19]. For IEF, ampholytes (LKB, Bromma, Sweden) of pI 3.5–10, 4–6 and 5–8 as 1:1:4 were used in sample buffer and gel. Samples were taken up in 8.8 M urea, 0.4% SDS, 5% 2-mercaptoethanol, 0.2% ampholytes. After 45 min, an equal volume of 8.8 M urea, 2% Nonidet-P40, 5% 2-mercaptoethanol, 2% ampholytes was added and electrophoresis performed. The second dimension consisted of a 5–7.5% SDS-polyacrylamide gel. Kodak XAR-5 film was used in combination with intensifier screens (Cronex Lightning Plus, Dupont Chemical Co., Newton, CT) for autoradiography of ¹²⁵I-labeled materials.

2.6 Peptide mapping

To perform two-dimensional peptide mapping, specific bands were cut out of gels that previously had been stained, destained and autoradiographed. The gel slices were washed in destain solution (acetic acid, ethanol, water as 7.5:20:72.5), then methanol, and lyophilized. Per gel slice, 25 µg trypsin (TPCK, 217 U/g, Worthington, Millipore Co., Freehold, NJ) was added in 0.05 M NH₄HCO₃ and slices were incubated for 16 h at 37°C. Trypsin digestion was repeated once. Combined samples were lyophilized, taken up in water and lyophilized again. The dry protein was dissolved in 50% pyridine and electrophoresis was performed on cellulose TLC plates (no. 5502, E. Merck, Darmstadt, FRG) in pyridine, acetic acid, water (1:10:89), pH 3.5. Chromatography was done in the second dimension with pyridine, acetic acid, n-butanol, water (10:3:15:12) as running fluid.

2.7 Lymphocyte proliferation assay

The antigen-induced lymphocyte proliferation assays were carried out in serum-free medium as described [20]. Two $\times 10^5$ LF

cells from donors primed with *Helix pomatia* hemagglutinin (HPH) or boosted with tetanus toxoid (TT) were cultured in triplicate in microtiter wells (Costar, Cambridge, MA, no. 3596) in the presence of HPH (10 µg/ml) or TT (1 LF/ml). These concentrations of antigens have been shown to be optimal in these tests [21]. Monocytes isolated by centrifugal elutriation were added in concentrations of 2×10^4 (10%) or 5×10^3 (2.5%). mAb were added in a final dilution of 1:200 and were present throughout the culture period. The cell mixtures were cultured in a final volume of 0.2 ml for 7 days at 37°C in a humidified atmosphere with 5% CO₂. Twenty µl containing 0.4 µCi = 14.8 kBq tritiated thymidine ([³H]dThd) was added during the last 4 h of incubation. The cultures were harvested with an automatic cell harvester, MASH-II, and counted as described previously [3].

2.8 Lymphocyte cultures for antibody production

The lymphocyte cultures for antibody synthesis were also carried out in serum-free medium. Three $\times 10^5$ LF cells obtained from donors primed with HPH or boosted with TT were cultured in 2-ml wells (Costar no. 3424). The purified monocytes were added in concentrations of 3×10^5 monocytes/well (10%). The cell mixtures were cultured in a final volume of 1.5 ml medium and contained PWM at a final concentration of 1:400 (v/v). They were incubated at 37°C in a humidified atmosphere of 5% CO₂ for 8 days. After this incubation period the supernatants were harvested and specific antibody production was measured in an indirect enzyme-linked immunosorbent assay (ELISA).

2.9 ELISA for antibody production *in vitro*

Specific antibody production in the lymphocyte cultures was measured in an ELISA as described elsewhere [22]. The bottoms of 0.2 ml microtiter wells (Sterilin, Teddington, GB, no. M23AR) were coated with HPH or TT. One hundred µl of supernatant was tested in duplo and compared to a serum standard diluted 1:400 and 1:800 for HPH and 1:600 and 1:1200 for TT. After an incubation period of 45 min at 37°C the wells were washed 3 times and 100 µl of peroxidase-conjugated rabbit anti-human IgA, IgG, IgM, λ and κ (Dako, Copenhagen, Denmark) was added. After incubation and washing, 100 µl 0.05 M phosphate-buffered (pH 5.6) o-phenylenediamine dihydrochloride (0.2 mg/ml) was added in the presence of 0.045% (v/v) H₂O₂. After 45 min incubation at 37°C the reaction was stopped with 1 N H₂SO₄ and the extinctions were measured in a multichannel spectrophotometer (Titertek). The absorbance values of the supernatants were compared with those of the serum standards. The antibody present in the supernatants was quantitated by probit analysis using the absorbance of the highest serum standard concentrations arbitrarily assigned a value of 100 Units/ml.

3 Results

3.1 Biochemical characterization of LFA-1 with different anti-LFA-1 mAb

It has been demonstrated recently that LFA-1, Mo-1 and p150,95 belong to a family of related human leukocyte membrane glycoproteins. These antigens consist of an α and β

chain which are noncovalently associated. The β chains of each of these antigens have been shown to be similar in MM and IEF pattern [8]. This implies that mAb directed against the β chain of LFA-1 also will precipitate Mo-1 and p150,95 from monocytes and granulocytes. To investigate whether the various mAb directed against LFA-1 were directed against the α or β chain of this molecular complex, immunoprecipitations were carried out with ¹²⁵I-labeled lysates of the monoblastic cell line KG-1. The mAb SPV-L1, SPV-L5, SPV-L7 and SPV-L11 precipitated 2 bands of 170 kDa (α chain) and 95 kDa (β chain) indicating that these antibodies were directed against the α chain. In contrast, mAb CLB54 and MHM-23 detected an additional band of 150 kDa (Fig. 1). The 165 kDa band is also precipitated from KG-1 (Fig. 2) but not detectable in a broad 170-kDa α chain of LFA-1 (Fig. 1). This is established in Fig. 3 in which it is shown that MHM-23 and CLB54 indeed precipitated four proteins from ¹²⁵I-labeled monocyte lysates of 170, 165, 150 and 95 kDa, respectively. Similar reaction patterns were observed by Springer et al. [8] with the mAb TS1/18 which was shown to be directed against the β chain of LFA-1. Therefore, our results indicate that CLB54 and MHM-23 are directed against the β chain of LFA-1. Since the β chains of LFA-1, Mo-1 and p150,95 are similar [8], the 165-kDa and 150-kDa bands precipitated from monocytes and

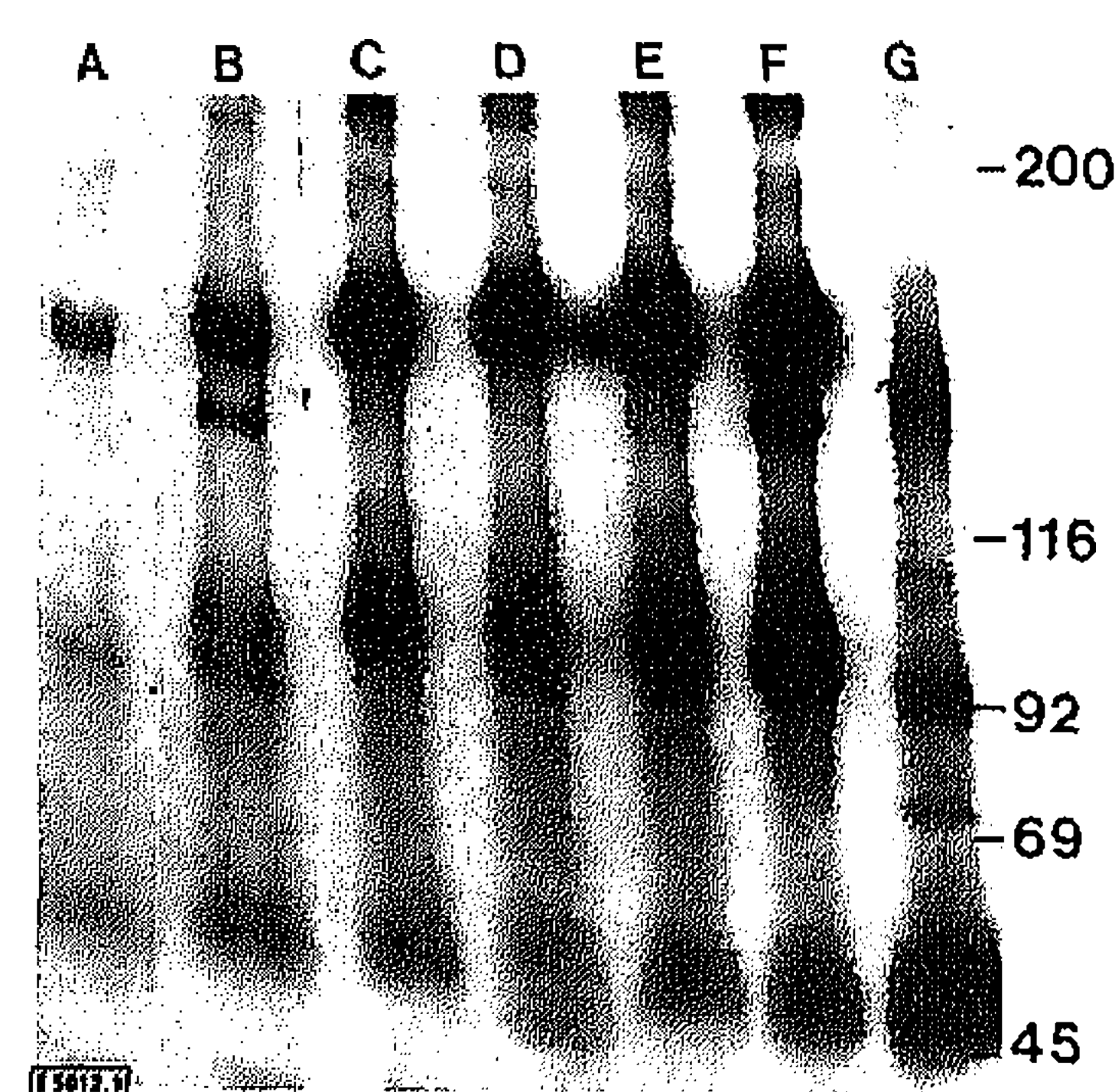


Figure 1. Precipitation of LFA-1 from a ¹²⁵I-labeled lysate of the monoblastic cell line KG-1 with various anti-LFA-1 antibodies. The lysate was precleared three times with rabbit anti-mouse-normal mouse serum preformed complex (RAM-NMS pfc) and divided into six equal portions, followed by immunoprecipitation with pfc of the antibodies mentioned below. Immunoprecipitates were subjected to SDS 7.5% PAGE and autoradiography. (A) SPV-L1; (B) CLB54; (C) SPV-L5; (D) SPV-L7; (E) SPV-L11; (F) MHM-23; (G) RAM-NMS pfc (control).

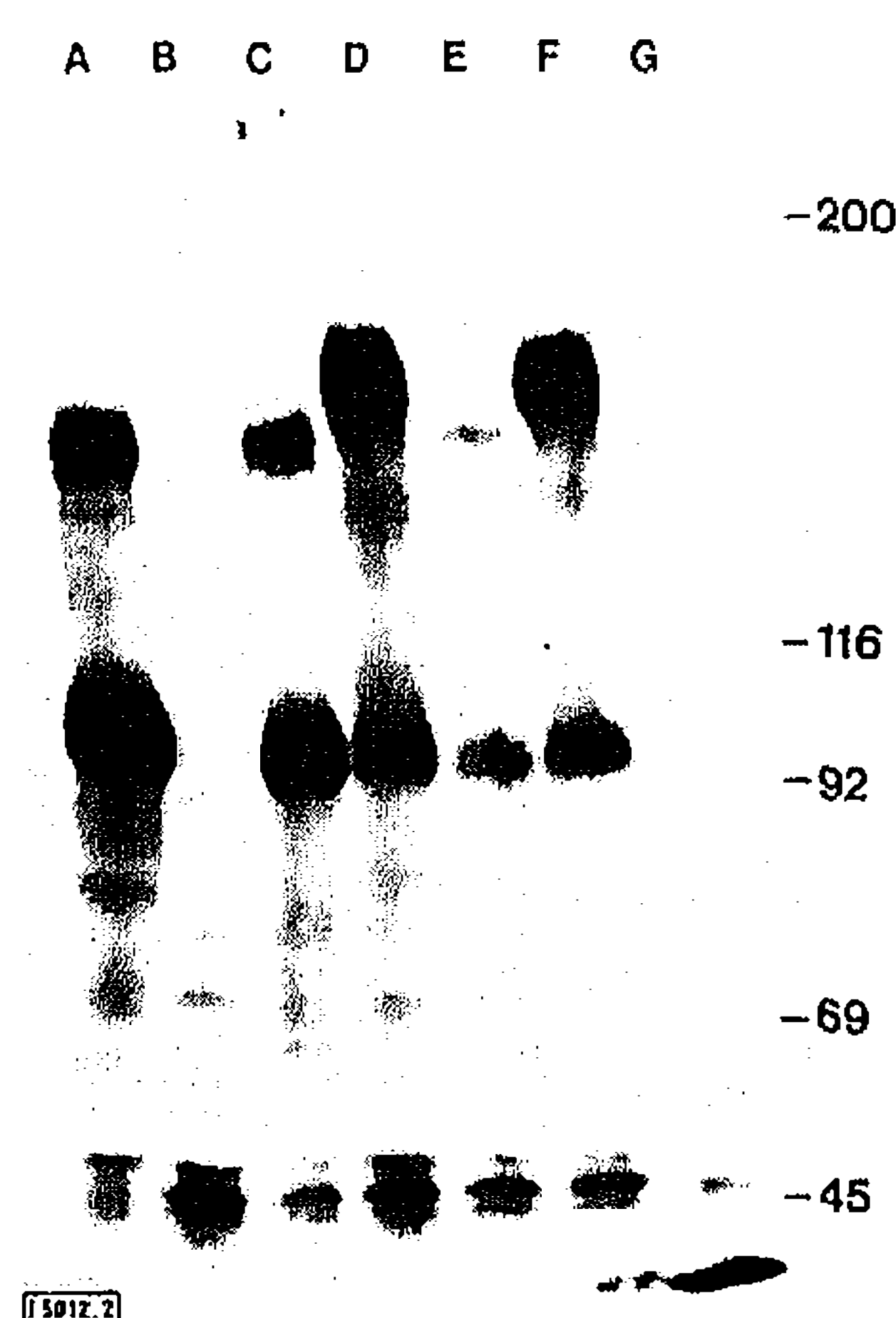


Figure 2. Precipitation of LFA-1 and Mo-1 from ¹²⁵I-labeled lysates from unstimulated and TPA-stimulated KG-1 cells. Lysates were precleared repeatedly with nonspecific pfc followed by precipitation with specific pfc. (A) Bear-1 precipitated from a ¹²⁵I-labeled lysate of monocytes. (B) RAM-NMS pfc control, TPA-treated cells (TTC). (C) Bear-1 (recognizing Mo-1 antigen) TTC. (D) SPV-L7 (recognizing LFA-1 antigen) TTC. (E) Bear-1 from unstimulated cells (UC). (F) SPV-L7 (UC). (G) RAM-NMS pfc UC (control).

KG-1 cells represent the α chains of Mo-1 and p150,95, respectively. No differences in MM of the α and β chains of LFA-1 were observed, indicating that the LFA-1 antigens precipitated by the various anti-LFA-1 antibodies were not heterogeneous.

3.2 LFA-1 and Mo-1 on KG-1 cells induced to differentiate with TPA

KG-1 can be induced to differentiate to monocytes by the phorbol ester TPA [11]. Immunoprecipitations carried out with radiolabeled lysates of KG-1 cells before and after TPA-induced differentiation showed a strong increase in the α and β chains of Mo-1 (Fig. 2). These results confirm immunofluorescence data which demonstrated that the expression of Mo-1 was strongly enhanced after incubation with TPA (De Vries et al., manuscript in preparation). The low expression of Mo-1 on the nonstimulated KG-1 cells also explains why the Mo-1 α chain is difficult to detect in the immunoprecipitates obtained with CLB54 and MHM-23 from KG-1 cells shown in Fig. 1, whereas the LFA-1 α chain is present as a very strong band.

3.3 Biochemical characteristics of LFA-1 and Mo-1 on lymphocytes, granulocytes and monocytes

To investigate whether LFA-1 was heterogeneous on different cells derived from the same donor, immunoprecipitations were carried out with ^{125}I -labeled lysates of monocytes, granulocytes and lymphocytes, and the mAb SPV-L7, Bear-1 and CLB54. The α and β chains of LFA-1 and Mo-1 expressed on monocytes, granulocytes and lymphocytes containing null cells have identical MM indicating that LFA-1 and Mo-1 on the different cells are not heterogeneous (not shown). Interestingly, heterogeneity was observed in the MM of the α chain of p150,95 in the immunoprecipitates obtained with the mAb CLB54 (Fig. 3), which is directed against the β chain of LFA-1. The MM of the α chain of p150,95 expressed on monocytes is higher than that of the α chain expressed on granulocytes

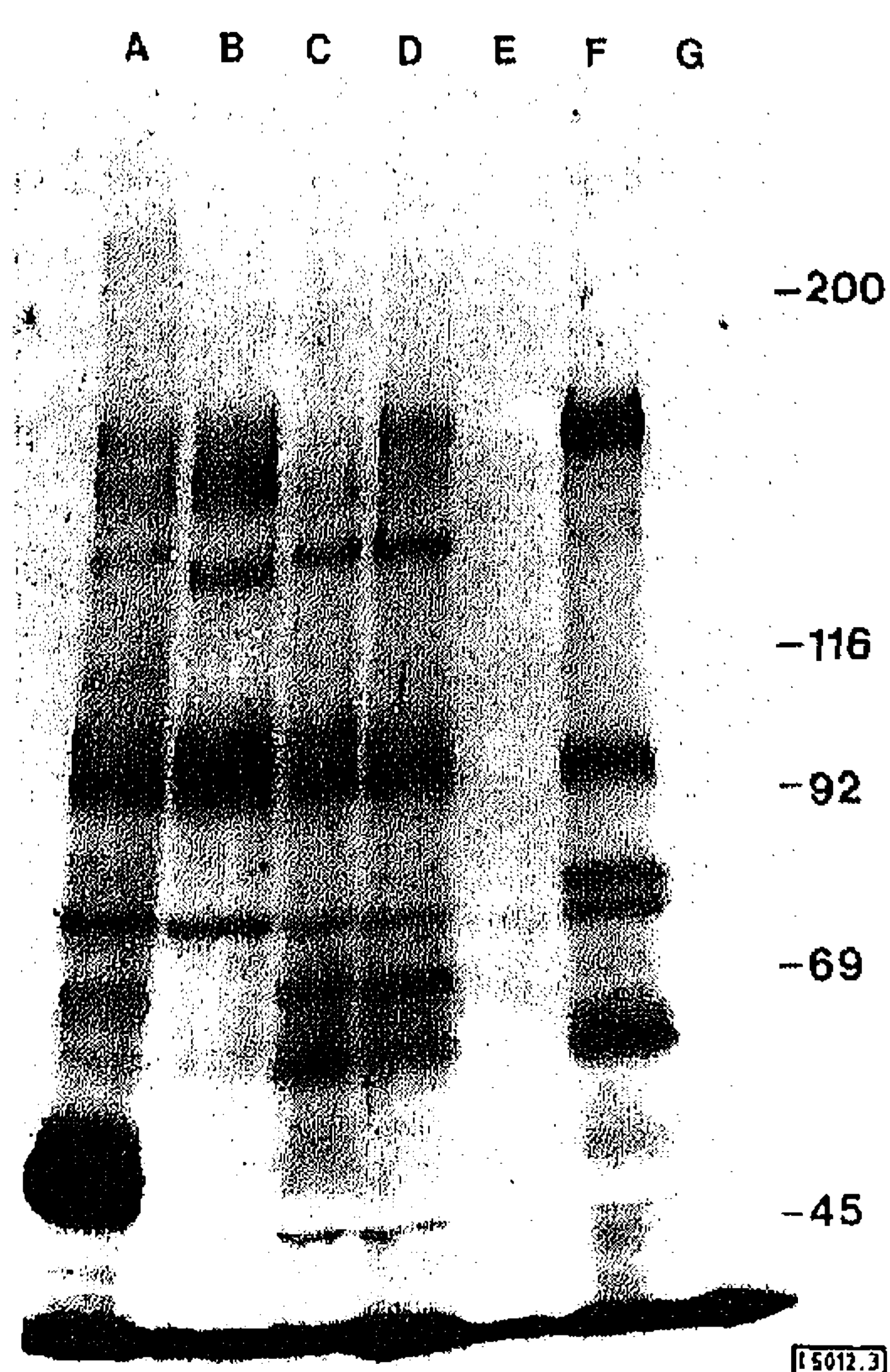


Figure 3. Precipitation with CLB54, which recognizes the common β chain, from ^{125}I -labeled monocytes, granulocytes and lymphocytes (containing "null" cells) derived from one donor. Lymphocytes, three monocyte subsets which differ in density and function [9], and granulocytes were obtained by centrifugal elutriation (purity >95%), labeled and precleared three times with RAM-NMS pfc. Subsequently, precipitations with CLB54 or MHM-23 (anti- β chain) were carried out, followed by washing and SDS 5-7.5% PAGE. For reasons unknown, one of the monocyte fractions labeled poorly with ^{125}I (lane E). (A) MHM-23; (B-F) CLB54 from granulocytes, three monocyte fractions and lymphocytes, respectively. (G) RAM-NMS as control.

from the same donor, where it was shown to precipitate a 140-kDa band.

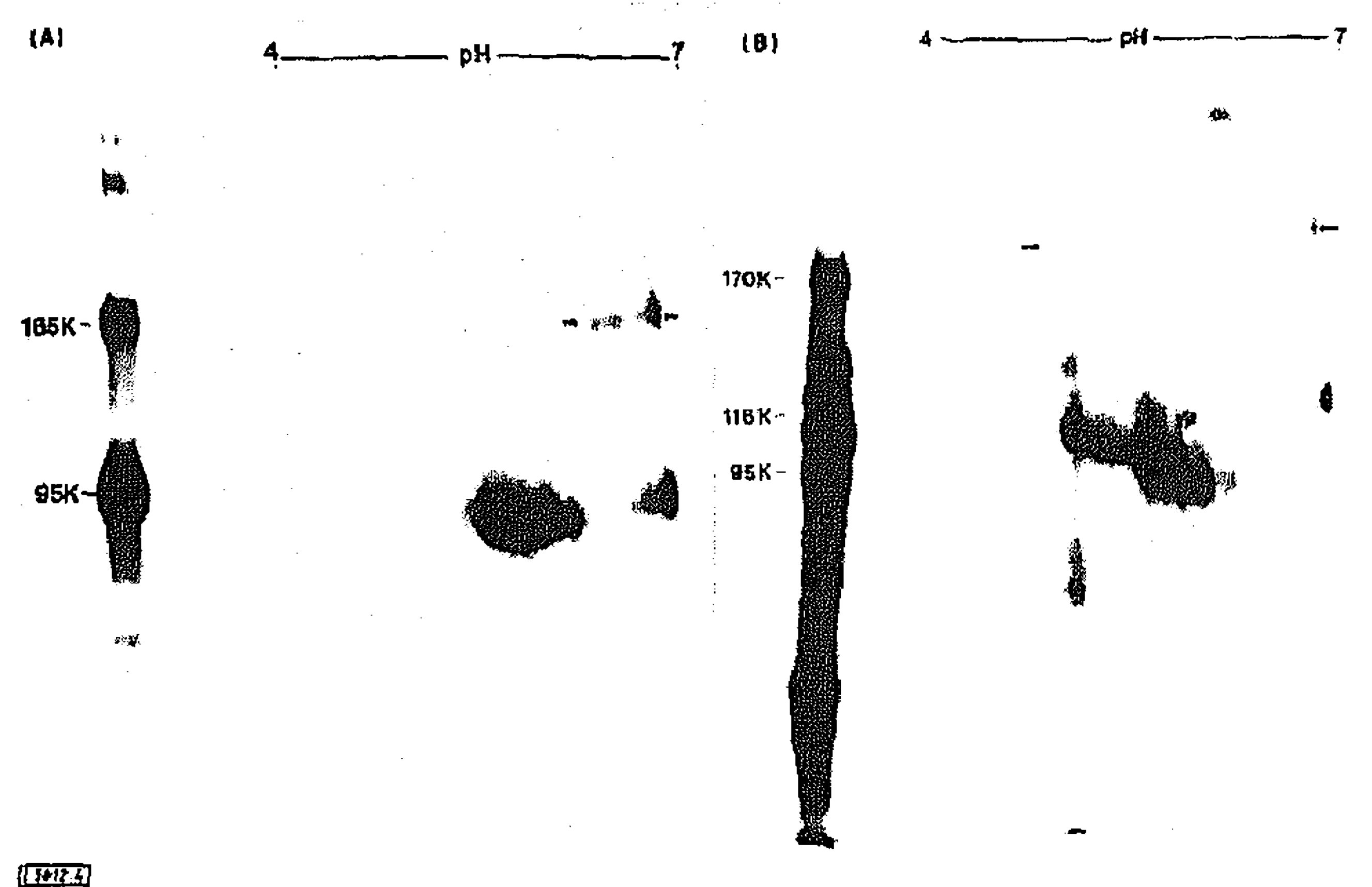


Figure 4. Two-dimensional gel electrophoresis of ^{125}I -labeled antigens of Mo-1 (A) and LFA-1 (B) detected by Bear-1 and SPV-L7, respectively, according to O'Farrell [18] using a 5-7.5% gel in the second dimension. The 110-kDa band observed in the immunoprecipitates of SPV-L7 is due to proteolytic breakdown of the LFA-1 α chain.

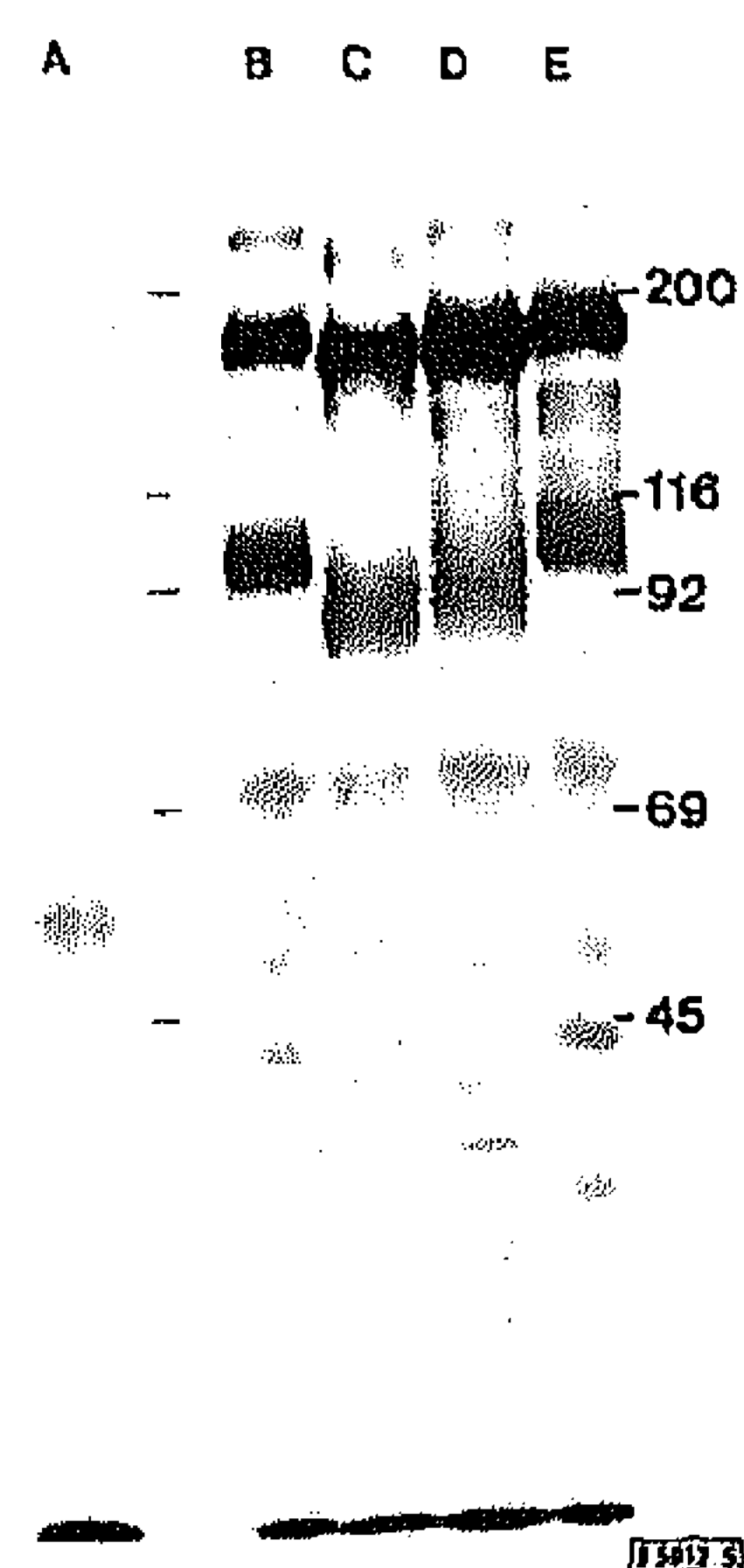


Figure 5. Analysis by SDS 5-7.5% PAGE of Mo-1 and LFA-1 treated with Endo-F. Monocytes were labeled with ^{125}I as described in Sect. 2.7, and Bear-1 and SPV-L7 immunoprecipitates were subjected to digestion with Endo-F (+), or incubated in the absence of enzyme (-). The proteins were recovered by precipitation with 10% trichloroacetic acid and analyzed on a 10% polyacrylamide gel. (A) RAM-NMS preclear; (B) Bear-1 (+); (C) Bear-1 (-); (D) SPV-L7 (+); (E) SPV-L7 (-).

from the same donor, where it was shown to precipitate a 140-kDa band.

3.4 Comparison of the molecular complexes detected by Bear-1 and anti-LFA-1

Sanchez-Madrid et al. [8] have shown a high degree of similarity between Mo-1 and LFA-1. Although the α chains of Mo-1 and LFA-1 had different MM of 170 and 165 kDa, respectively, the β chains were shown to be similar in MM and IEF pattern. We extended these results by demonstrating that the β chain of LFA-1 and Mo-1 [2, 14] (as detected by the mAb Bear-1 produced in our laboratory) not only had similar MM and IEF patterns (Fig. 4), but they were also identical as judged by their glycosylation and tryptic peptide mapping. Enzymatic digestion of the β chains of LFA-1 and Mo-1 with Endo-F, which cleaves off all N-linked sugars via hydrolysis of the glycosidic bond of the N-N-diacetylchitobiose core structure [17], resulted in an identical reduction in MM from 94 kDa to 90 kDa approximately (Fig. 5). These results indicated that the degree of N-linked glycosylation of both β chains is identical and that both β chains are equally sensitive to Endo-F treatment. In contrast Endo-F treatment of the α chains resulted in reaction products of different MM, indicating that the differences in the α chains were not related to differences in N-linked glycosylation.

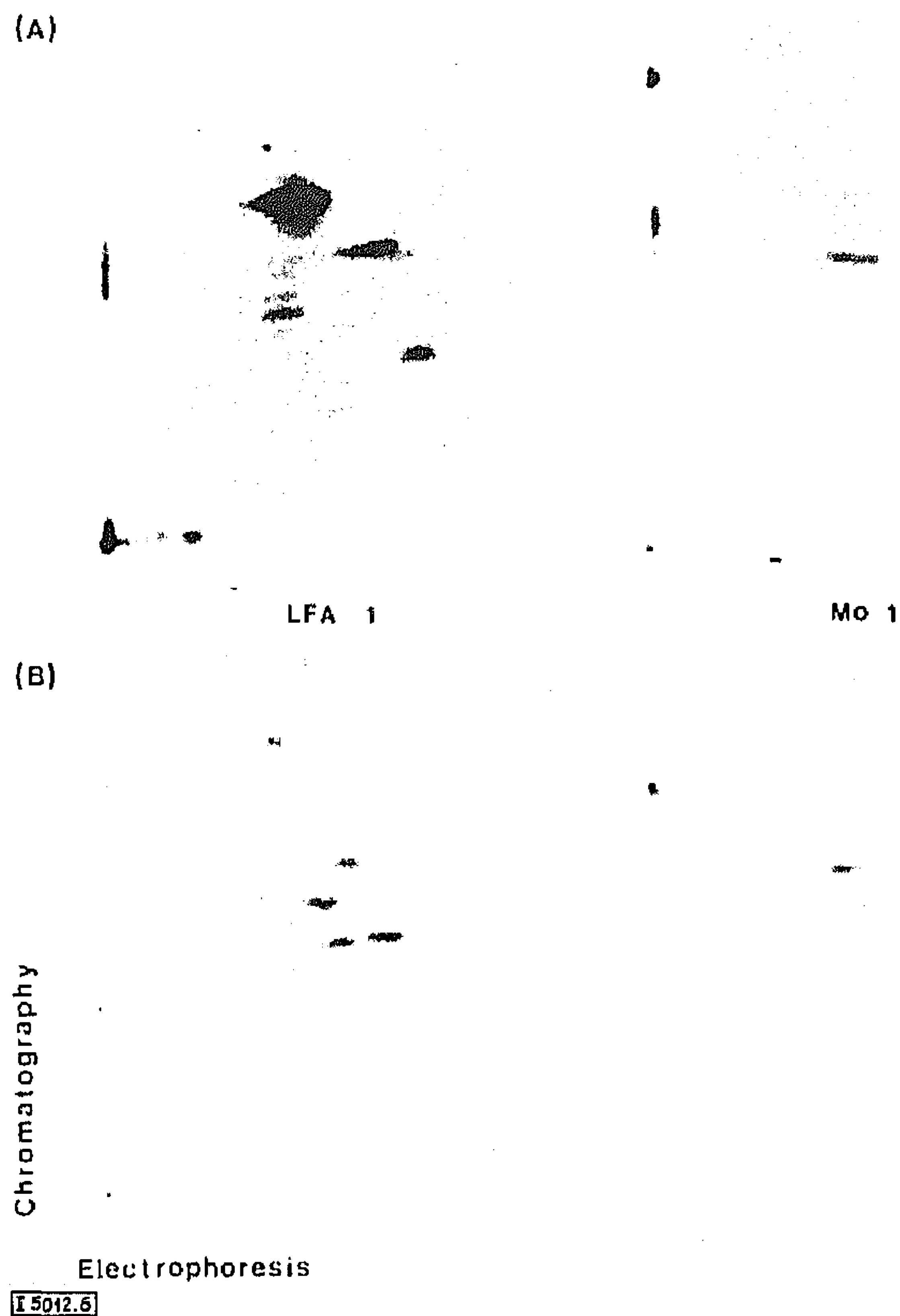


Figure 6. Tryptic peptide map of ^{125}I -labeled antigens of Mo-1 and LFA-1. Specific bands cut out from autoradiographed gels were treated as described in Sect. 2.6. (A) LFA-1 α (left); Mo-1 α (right); (B) LFA-1 β (left); Mo-1 β (right).

To establish that the protein backbones of the β chains of LFA-1 and Mo-1 are identical, tryptic peptide maps of both β chains were prepared. In Fig. 6 it is shown that the peptide composition of the β chains of both antigens are identical, whereas that of the α chains differed completely.

3.5 Biological properties of Mo-1 and LFA-1

Mo-1 and LFA-1 have similarities in biochemical structure and identical β chains. In addition it has been shown that both molecules act as adhesion molecules on granulocytes [7, 23, 24]. However, Mo-1 and LFA-1 have different α chains and are distributed on different cell types. Therefore, we investigated whether these antigens differed in other biological functions. Since LFA-1 and Mo-1 are both expressed on human monocytes, the effects of SPV-L5 and Bear-1 on monocyte-dependent reactions, like antigen-specific lymphocyte proliferation and PWM-driven specific antibody production *in vitro*, were investigated [21]. It was shown that both processes are monocyte dependent. Antigen-specific lymphocyte proliferation was strongly reduced after the monocytes had been depleted by centrifugal elutriation (Table 1). The proliferative responses were completely restored by the addition of 10% autologous monocytes. The PWM-driven specific antibody production was completely absent after removal of the monocytes; however, also in this situation specific antibody production could be restored to control levels by the addition of 10% autologous monocytes (Table 1). Interestingly, the antigen-specific proliferative response and the PWM-driven specific antibody production *in vitro* were inhibited by mAb directed against LFA-1, whereas Bear-1 and anti-Mo-1 had no effect

Table 1. Monocyte dependence of antigen-induced lymphocyte proliferation and specific antibody production after PWM stimulation^{a)}

Cells of donor no.	^3H dThd incorporation (cpm $\times 10^{-3}$)			Specific antibody production (U/ml)		
	PBL	Lymphocytes	Lymphocytes + monocytes	PBL	Lymphocytes	Lymphocytes + monocytes
1	25.1 \pm 4.1	0.6 \pm 0.2	21.0 \pm 4.3	40.6 \pm 5.2	0.1 \pm 0.1	44.7 \pm 6.2
2	33.3 \pm 6.2	2.0 \pm 0.6	39.9 \pm 0.7	NT ^{b)}	NT	NT
3	8.1 \pm 1.2	1.0 \pm 0.2	6.1 \pm 0.9	NT	NT	NT
4	NT	NT	NT	16.0 \pm 2.1	0.2 \pm 0.1	13.7 \pm 1.8

a) Four different donors were immunized with TT (donor 1 and 2) or HPH (donor 3 and 4). Several days (19–60) after immunization, specific lymphocyte proliferation or specific antibody production after PWM stimulation against TT or HPH was measured. Lymphocytes were depleted of monocytes by centrifugal elutriation [9], and contained less than 0.1% monocytes as judged by staining for nonspecific esterase. The amount of surface Ig⁺ cells was 5% (donor 1) and 7% (donor 4). Monocytes were added in a final concentration of 10%.

b) NT = Not tested.

Table 2. Effects of mAb against LFA-1 and Mo-1 on antigen-induced lymphocyte proliferation^{a)}

Donor	mAb:	^3H dThd incorporation (cpm $\times 10^{-3}$)				
		–	SPV-L5	Bear-1	Q5/13	SPV-L3
1		21.0 \pm 4.3	14.5 \pm 0.9	27.2 \pm 5.2	11.5 \pm 5.2	NT
2		39.9 \pm 0.7	4.0 \pm 2.8	43.2 \pm 4.2	2.5 \pm 1.3	12.9 \pm 5.0
3		6.1 \pm 0.9	0.7 \pm 0.2	5.9 \pm 1.9	1.5 \pm 0.4	1.2 \pm 0.2
% Inhibition		–	30–90	<5	45–95	65–80

a) Three different donors were immunized with TT (donor 1 and 2) or HPH (donor 3). *In vitro* stimulation of lymphocytes supplemented with 10% autologous monocytes resulted in lymphocyte proliferation. The mAb SPV-L5 (anti-LFA-1 α), Bear-1 (anti-Mo-1 α), Q5/13 (anti-HLA-DR), SPV-L3 (anti-HLA-DC) and Rup-1

(35-kDa myeloid differentiation antigen) were added to a final concentration of 1:200 of ascites.

Table 3. Effects of mAb against LFA-1 and Mo-1 on the PWM-driven specific antibody production^{a)}

Donor	mAb:	Specific antibody production (U/ml)				
		-	SPV-L5	Bear-1	Q5/13	Rupi-1
1		44.7 ± 6.3	0.8 ± 0.2	40.4 ± 7.1	0.1 ± 0.1	0.2 ± 0.1
4		13.7 ± 1.8	0.4 ± 0.1	13.1 ± 2.4	1.4 ± 0.3	0.8 ± 0.3
% Inhibition		-	>95	<10	>90	>95

a) Two different donors were immunized with TT (donor 1) or HPH (donor 4). *In vitro* stimulation of lymphocytes supplemented with 10% autologous monocytes with PWM resulted in antibody production against TT (donor 1) or HPH (donor 4). The antibody production was measured with an ELISA (see Sect. 2.9). For mAb used see Table 2.

(Tables 2 and 3). Furthermore, it is shown that the mAb Q5/13 (anti-HLA-DR) and SPV-L3 (anti-HLA-DC) completely inhibited the antigen-specific T cell proliferation and antibody production *in vitro*. The mAb Rupi-1 which detects a 35-kDa differentiation antigen only present on human monocytes and granulocytes was ineffective (Tables 2 and 3). These results indicate that in spite of their identical β chains, LFA-1 and Mo-1 differ in their biological functions. LFA-1 is apparently associated with monocyte-T cell interaction, whereas Mo-1 is not.

4 Discussion

In this study the biochemical and biological characteristics of LFA-1, Mo-1 and p150,95 were investigated. All three antigens are only expressed on human monocytes, granulocytes and "null" cells, whereas LFA-1 is also present on human thymocytes and on resting and activated T and B cells [1, 2]. The monocyte differentiation antigen Mo-1, which was characterized as the receptor for the inactivated form of C3b, was analyzed with a mAb Bear-1. Precipitations of LFA-1 from monocytes, granulocytes and KG-1 carried out with 6 different mAb directed against LFA-1 showed that all 6 antibodies precipitated the same bimolecular complex with an α chain of 170 kDa and a β chain of 95 kDa from KG-1 cells. Four of these antibodies (SPV-L1, SPV-L5, SPV-L7 and SPV-L11) were directed against the α chain. The two other mAb (CLB54 and MHM-23) were directed against the β chain of LFA-1, which was reported to be similar to the β chains of Mo-1 and p150,95 [8]. Therefore, the immunoprecipitates of these antibodies and radiolabeled lysates of monocytes, granulocytes and KG-1 also contained the 165-kDa α chain of Mo-1 and the 150-kDa chain of p150,95. In addition, immunoprecipitations carried out with SPV-L7 (anti-LFA-1 α chain), Bear-1 (anti-Mo-1 α chain) (both not shown), CLB54 (anti-LFA-1 β chain) and radiolabeled lysates of lymphocytes, monocytes and granulocytes isolated from the same donor revealed no heterogeneity in the α chains of LFA-1 and Mo-1 expressed on the various cell types. Interestingly, the α chain of the p150,95 molecule was found to be heterogeneous. The p150 α chain on monocytes was consistently found to have a higher MM than the α chain on granulocytes derived from the same donor. The nature of these differences in MM of p150 α chains remains to be determined. However, recently we observed that the p150,95 glycoprotein is associated with the chemotaxis of human monocytes and the adherence of these cells to plastic surfaces and substrates consisting of endothelial cells*. In contrast to their association with adherence of granulocytes, LFA-1 and Mo-1 were not involved in the adherence of monocytes*. Sanchez-Madrid et al. [8] were the first to demonstrate that

the β chains of LFA-1 and Mo-1 had similar MM and IEF patterns. We extended these data by establishing that the β chains of these antigens had exactly the same degree of N-linked glycosylation and that both β chains were equally sensitive to Endo-F treatment. The differences in MM of the α chains could not be attributed to differences in N-linked glycosylation because after Endo-F treatment the digestion products still differed significantly in MM. The similarity of the β chains was confirmed further by tryptic peptide maps which showed that the β chains of LFA-1 and Mo-1 also had identical protein backbones. In contrast, the protein backbones of both α chains were completely different. In spite of their structural similarities and the finding that both molecules when expressed on granulocytes act as adhesion molecules [7, 24], Mo-1 and LFA-1 differ in other biological functions.

It is shown that LFA-1 is also required for monocyte-T cell interactions. Monocyte-dependent specific proliferative T cell responses to the soluble antigens TT or HPH were abrogated strongly by mAb against LFA-1. Interestingly, anti-LFA-1 antibodies also completely inhibited the PWM-driven specific antibody production to HPH and TT *in vitro*. In contrast, Bear-1 and anti-Mo-1 had no effect on antigen-specific lymphocyte proliferation or specific antibody production *in vitro*. Thus, in spite of its identical β chain, Mo-1 was not associated with monocyte-T helper cell interaction.

LFA-1, Mo-1 and p150,95 are related structurally. All 3 molecules are also associated with adhesion functions of granulocytes. On the other hand, the present findings indicate that LFA-1 and Mo-1 are completely different in the biological functions described here. These differences may be related to their different α chains, which may recognize specific counter-structures. Recent studies carried out in patients who are deficient for LFA-1, Mo-1 and p150,95 have shown that the identical β subunit seems to be required for the surface expression of the α - β heterodimeric complexes [8, 24].

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Short papers

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Biosynthesis of complement protein D by HepG2 cells: a comparison of D produced by HepG2 cells, U937 cells and blood monocytes*

The biosynthesis of complement protein D of the alternative pathway by HepG2 cells, a human hepatocyte cell line, was studied and compared to the biosynthesis of D by U937 cells and blood monocytes. Increasing amounts of antigenic D were detected in HepG2 cell culture supernatants by radioimmunoassay. The kinetics of D synthesis and secretion by HepG2 cells was followed in a pulse-chase study using [³⁵S]cysteine. As analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by autoradiography, only a single D band was seen intra- and extracellularly and both forms had the same apparent molecular weight as D synthesized by U937 cells or purified from serum. Treatment of HepG2 and U937 cells with canavanine, an arginine amino acid analog, to inhibit intracellular processing resulted in slight depression of the apparent molecular weight of D synthesized by these cells. D synthesized by blood monocytes had an apparent molecular weight similar to that synthesized by HepG2 and U937 cells, suggesting that these cell lines do not synthesize and process D differently than normal monocytes. The data demonstrate that the hepatocyte is a site of D synthesis and suggest that D is not synthesized as a precursor molecule.

[I 5113]

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Abbreviations: PBS: Phosphate-buffered saline FCS: Fetal calf serum SDS: Sodium dodecyl sulfate PAGE: Polyacrylamide gel electrophoresis

1 Introduction

Studies on the biosynthesis of complement proteins (reviewed in [1]) have shown that the liver and cells of the monocyte/macrophage series are the major sites of synthesis, although other cell types also synthesize certain complement proteins [2, 3]. Complement protein D of the alternative pathway has been the subject of limited biosynthetic study [4–6], however, recent work has demonstrated that U937 cells, a human monocyte cell line, synthesize D [7]. Several questions were raised by this study, concerning the existence of a biosynthetic precursor, tissue specific differences in the post-translational processing and synthesis of D by hepatocytes.